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(57) Abstract

An electrophoretic method for purifying a nucleic acid sample is disclosed. The method generally comprises the steps of (1) providing a nucleic acid sample comprising a desired nucleic acid and one more contaminants, (2) providing an electrophoresis matrix having a loading well and a recovery well formed therein, (3) placing the nucleic acid sample into the loading well, (4) performing a first electrophoresis comprising electrophoresing the nucleic acid sample for a first time effective to transport the desired nucleic acid out of the loading well and into the electrophoresis matrix; and (5) performing a second electrophoresis comprising electrophoresing the nucleic acid sample for a second time effective to transport the desired nucleic acid out of the electrophoresis matrix and into the recovery well. According to the method, the first and second electrophoresis steps are effective to substantially reduce the concentration of contaminants relative to the concentration of desired nucleic acid in the nucleic acid sample, thereby producing a purified nucleic acid. In the method, the loading and recovery wells may be the same or different, and the electric fields may be DC or alternating. Also disclosed is a preparative electrophoresis method employing an alternating electrical field.

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ELECTROPHORETIC NUCLEIC ACID PURIFICATION METHOD

FIELD OF THE INVENTION

This invention relates to the field of electrophoresis. More specifically, this invention relates to electrophoretic methods and apparatus useful for the purification of nucleic acids.

BACKGROUND

Analysis of nucleic acid structure has become the focus of much of modern biology, biotechnology and medicine. Modern nucleic acid analysis techniques such as PCR, fragment-length-polymorphism analysis, and DNA sequencing provide information useful for a variety of applications including diagnosis of disease, organism identification, and tracking evolutionary relatedness. A necessary preliminary step in any nucleic acid analysis method is the preparation of nucleic acid which is free from contaminants which can interfere with enzymes used in these techniques, e.g., contaminants which can inactivate polymerase enzymes used in PCR and DNA sequencing methods.

A wide variety of nucleic acid purification techniques are available based on a range of different physical and chemical principles. The most common nucleic acid purification methods include organic/aqueous liquid-liquid extraction, solid-phase adsorption, precipitation, density-gradient centrifugation, and preparative electrophoresis. Electrophoretic methods are particularly attractive because they result in nucleic acid having a high purity and a large molecular weight.

But, conventional preparative electrophoretic methods suffer from significant shortcomings which limit their practical utility, particularly in the context of high-throughput applications. A particularly problematic aspect of conventional preparative electrophoretic methods is the manner in which a purified nucleic acid is removed from an electrophoresis medium, e.g., an electrophoresis gel. In one class of sample removal processes, the purified nucleic acid is manually

excised from the electrophoresis gel. These sample-excision methods are disadvantageous because the nucleic acid and gel material must be separated after excision of the sample band, the procedure requires significant manual intervention, and the purified nucleic acid must be visualized prior to excision in order to locate the desired sample band. In a second class of sample removal techniques, the purified sample is eluted off of the electrophoresis gel into a gel-free buffer. However, such elution methods require that multiple fractions be collected, the purified sample band be visualized, and/or the elution properties of the desired nucleic acid be known.

SUMMARY

The present invention is directed towards the discovery of a class of novel preparative electrophoresis methods useful for the purification of nucleic acids. The methods are particularly useful for the preparation of nucleic acid samples prior to treatment with enzymes, e.g., in Sanger-type sequencing, oligonucleotide ligation assays, and PCR.

In a first aspect, the invention comprises a method for purifying a nucleic acid sample comprising the steps of (1) providing a nucleic acid sample comprising a desired nucleic acid and one or more contaminants, (2) providing an electrophoresis matrix having a loading well and a recovery well formed therein, placing the nucleic acid sample into the loading well, (3) performing a first electrophoresis comprising electrophoresing the nucleic acid sample for a first time effective to transport the desired nucleic acid out of the loading well and into the electrophoresis matrix, (4) performing a second electrophoresis comprising electrophoresing the nucleic acid sample for a second time effective to transport the desired nucleic acid out of the electrophoresis matrix and into the recovery well, (5) wherein the first and second electrophoresis is effective to substantially reduce the concentration of contaminants relative the concentration of desired nucleic acid, thereby producing a purified nucleic acid. In the method, the loading well and the recovery well may be the same well or different wells.

In a first preferred embodiment of this first aspect of the invention, referred to herein as the "trap mode" of the invention, the loading well and the recovery well are spatially overlapping wells, and the electrophoresis matrix comprises a bulk portion and a well-matrix-interface portion, and the matrix is effective to trap the desired nucleic acid in the well-matrix-interface portion such that the desired nucleic acid is substantially prevented from entering the bulk portion of the matrix.

In a second preferred embodiment of the first aspect, referred to herein as the "contaminant-dilution mode" of the invention, the loading well and the recovery well are spatially overlapping wells, and the first electrophoresis is sufficient to transport a portion of the contaminants out of the loading well, through the electrophoresis matrix, and into a contaminant dilution reservoir, the reservoir containing a volume of buffer sufficient to substantially dilute the contaminants entering the reservoir.

In a third embodiment of the first aspect of the invention, referred to herein as the "LITAC-reverse-field mode", the loading well and the recovery well are spatially overlapping wells, the first electrophoresis employs a DC electrical field and the second electrophoresis employs a LITAC electrical field.

In a fourth preferred embodiment of the first aspect present invention, the loading and recovery wells are spatially distinct, and the first electrophoresis employs a DC electrical field and the second electrophoresis employs a LITAC electrical field.

In a fifth preferred embodiment of the first aspect present invention, the loading and recovery wells are spatially distinct, and the first electrophoresis comprises electrophoresing the desired nucleic acid in a first direction and the second electrophoresis comprises electrophoresing the desired nucleic acid in a second direction different from the first direction.

In a second aspect, the present invention consists of a method for the electrophoresis of a nucleic acid sample located in an electrophoresis matrix comprising subjecting the nucleic acid sample to a LITAC electrical field comprising a forward electrical field E_F and a reverse electrical field E_R.

In a third aspect, the present invention comprises a method for the electrophoresis of a nucleic acid sample located in an electrophoresis matrix comprising subjecting the nucleic acid sample to a ZIVE electrical field comprising a forward electrical field E_F and a reverse electrical field E_R.

Various aspects and/or embodiments of the above-described invention achieve one or more of the following important advantages over known electrophoretic purification methods: (1) using the methods of the invention, there is no need to physically remove sample bands from an electrophoresis gel subsequent to electrophoretic separation--instead, the purified sample is located in a gel-free recovery well and is dissolved in a buffer suitable for subsequent enzymatic treatment, thereby greatly facilitating the automation of post-purification sample recovery; (2) using the methods of the invention, there is no need to collect multiple fractions resulting from a post-electrophoresis elution process--instead, the purified sample is located in a gel-free recovery well and is dissolved in a buffer suitable for subsequent enzymatic treatment, thereby reducing the amount of sample dilution, eliminating the need to collect multiple fractions, and eliminating the requirement for *a priori* knowledge of the migration behavior of a desired nucleic acid; (3) using the methods of the invention, there is no need to visualize the desired nucleic acid subsequent to electrophoretic separation in order to effect its recovery--instead, subsequent to electrophoresis, the purified sample is located in a gel-free recovery well; (4) using the methods of the invention, there is no need to separate an electrophoresis matrix from a purified nucleic acid; and, (5) using the methods of the invention, nucleic acid is purified to a sufficient purity to allow for efficient PCR amplification in a single step without the need to perform centrifugation or ethanol precipitation to concentrate the purified nucleic acid.

These and other features and advantages of the present invention will become better understood with reference to the following description, drawings, and appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1E show a schematic depiction of the contaminant-dilution embodiment of the invention.

FIGS. 2A-2D show a schematic depiction of a trap embodiment of the invention.

FIGS. 3A-3C show a schematic depiction of a LITAC reverse field embodiment of the invention.

FIGS. 4A-4D show a schematic depiction of a single direction multi-well embodiment of the invention.

FIGS 5A-5D show a schematic depiction of a multi-direction multi-well embodiment of the invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Reference will now be made in detail to the preferred embodiments of the invention, examples of which are illustrated in the accompanying drawings. While the invention will be described in conjunction with the preferred embodiments, it will be understood that they are not intended to limit the invention to those embodiments. On the contrary, the invention is intended to cover alternatives, modifications, and equivalents, which may be included within the invention as defined by the appended claims.

Generally, the methods of the present invention effect the separation of a nucleic acid sample into two fractions: a first fraction comprising nucleic acid

molecules smaller than a critical size, M*, and contaminants which interfere with enzymatic treatment of a purified nucleic acid, and a second fraction comprising nucleic acid molecules larger than M*, such molecules being referred to herein as a "desired nucleic acid".

The methods of the invention generally comprise the following six method steps: (1) providing a nucleic acid sample comprising a desired nucleic acid and one or more contaminants; (2) providing an electrophoresis matrix having a loading well and a recovery well formed therein, wherein the loading and recovery wells may be the same well or different wells; (3) placing the nucleic acid sample into the loading well; (4) performing a first electrophoresis wherein the nucleic acid sample is electrophoresed for a first time effective to transport the desired nucleic acid out of the loading well; (5) performing a second electrophoresis wherein the nucleic acid sample is electrophoresed for a second time effective to transport the desired nucleic acid into the recovery well; and (6) removing the purified nucleic acid from the recovery well. The first and second electrophoresis steps are effective to substantially reduce the concentration of contaminants relative the concentration of desired nucleic acid in the nucleic acid sample, thereby producing a purified nucleic acid.

For ease of discussion, the methods of the invention are divided into two categories: single-well methods in which the loading well and the recovery well are the same well, and multi-well methods, in which the loading and recovery wells are spatially distinct.

I. GENERAL CONSIDERATIONS

A. Nucleic Acid Sample

The nucleic acid sample of the invention may be derived from any living or dead biological organism. Exemplary sources of sample nucleic acid include but are not limited to cells, microorganisms, tissue, blood, and viruses.